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Morphological, anatomical and histological studies on the olfactory organs and eyes of teleost fish: *Anguilla anguilla* in relation to its feeding habits

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Abstract The morphology, anatomy and histology of the olfactory organ of *Anguilla anguilla* have been described. It was found that each olfactory chamber opens externally by an anterior inlet and posterior outlet nostrils. The olfactory rosette situated in each chamber is oval and the number of its olfactory lamellae in the olfactory rosette increases with the increase of body length. The olfactory epithelium of the lamellae is composed of receptor, supporting, basal and goblet cells.

The average olfactory surface area is about 590.9% of the retinal area. Thus *A. anguilla* is a macrosmatic species "nose-fish" in which olfaction plays an important role in its feeding habit. It is noticed that, the photoreceptor cell layer in *A. anguilla* (bottom feeder) is made up only of rod cells.

Contents

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Introduction

The feeding habits of fishes are reflected on the structure and size of sense organs, particularly the eyes and olfactory organs. For many animal groups vision is the most important, while information about the chemical nature of the external environment is more essential in others. For fishes, where life is entirely restricted to the aqueous environment, chemoreception plays a major and sometimes decisive role in many of its behaviour such as feeding, defence, spawning, schooling, orientation and migration (Hara, 1975). Nevertheless, according to Popova (1967) predaceous fish may be divided into two groups on the basis of their method of finding and procuring food; being diurnal and nocturnal predators. In the former group vision plays the main role in capturing prey, whereas nocturnal fishes apply the senses of smell, touch and lateral line organs. Burne (1909) and Teichmann (1954) had earlier classified the olfactory rosettes of fishes into three specific morphological types. Fishes having greater number of olfactory lamellae show behavioural responses to olfactory stimulation, nose fish (macrosmatic). But, fishes having lesser number of lamellae, show lesser response to olfaction and greater to sight, eye fish (microsmatic). The intermediate between macrosmatic and microsmatic, mediosmatic ones, eye-nose fishes. The literatures on the structure of the olfactory organs in many teleostean fishes have recently been reviewed extensively by (El-Attar and El-Agamy, 1989; El-attar, 1990; El-Agamy and El-attar, 1990, 1991; El-Attar et al., 1999, 2006, 2010; Kumari, 2008; Zeiske et al., 2009 and Charkrabari and Gosh, 2010).

The visual system in teleostean fishes has attracted the attention of many authors such as (El-Attar et al., 1999; Donatti and Fanta, 2002, 2007; Asli et al., 2012 and Begum et al., 2013).

The present work aimed to find out a correlation between the olfactory organ and retina on one side and the feeding habits on the other side in the teleost fish *A. anguilla*.

Materials and methods

Adult 26 live specimens of *A. anguilla* (Family: Anguillidae) were collected from Bahr Mouas, a branch of the River Nile in Sharkia province. The standard length of specimens was ranged from 34.2 to 54.4 cm.

For the morphological and morphometric studies, the fish specimens were immediately sacrificed. The fish head then was fixed in 10% formalin in order to be dissected from their cranial roof. However, for histological studies, the olfactory organs were fixed in Bouin's solution and eyes in Carnoy's fluid. Transverse and vertical serial sections of 6 μm thick, were stained with Harris haematoxylin and Toluidine blue.

Morphometric studies

For morphometric studies, each of the following parameters was measured in cm (SL = standard length).

Area of retina

The area of retina (SR) was calculated by using the equation: $SR = 2\pi r^2$ where (π) is constant and (r) is the average of the two radii of the eye.

Olfactory area

The olfactory rosettes of both sides were dissected out carefully, and the number of lamellae in each rosette is counted. Then after, the left and right rosette were stained for 10 min with neutral red the olfactory lamellae were separated from the mother rosette, mounted in glycerine and were drawn by camera lucida to illustrate their measurements. The average olfactory surface area of all lamellae in each fish was calculated for one rosette, and compared with the area of retina according to the methods described by Teichmann (1954).

The obtained data were recorded in tables, analysed and curves were made to reveal the relationship between these parameters.

Results

The olfactory organs

In *A. anguilla* the olfactory organs are represented by a pair of olfactory chambers situated dorso-anteriorly between the eyes and snout. Each olfactory chamber opens externally by two nostrils; an anterior inlet and a posterior outlet and is occupied by an olfactory rosette, formed of numerous lamellae (Fig. 1).

Dissection of the head from the dorsal side shows the anatomical relationship between the brain and the olfactory rosette. The olfactory lobes are closely situated to the cerebrum so the olfactory nerves are long and thick. Each nerve is formed of bundles of olfactory fibres arising from the ventral surface of the corresponding rosette (Fig. 2).

It has been found that the olfactory rosette is oval in shape and occupies most of the olfactory chamber cavity. Thus, it belongs to type II of Bateson (1889) or column II of Burne

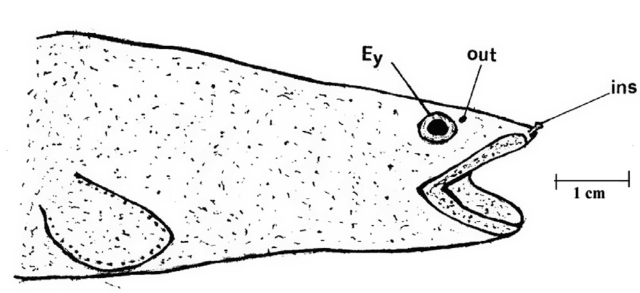

Figure 1 Camera, lucida drawing of dorsal view of the head of *A. anguilla*, showing Ey, eye; out, outlet nostril; ins, inlet nostril.

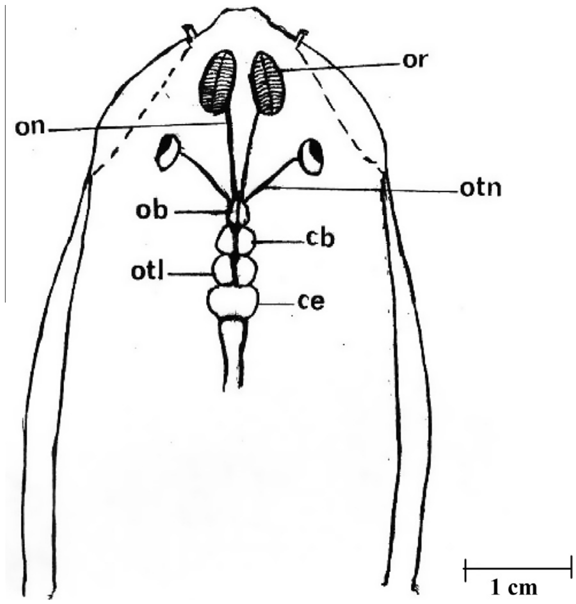

Figure 2 Dissection of the head from the dorsal side showing the relationship of the brain and the olfactory rosettes of *A. anguilla*, or, olfactory rosette; on,olfactory nerve; ob, olfactory lobe; ce, cerebellum; cb, cerebrum; otn, optic nerve; otl, optic lobe.

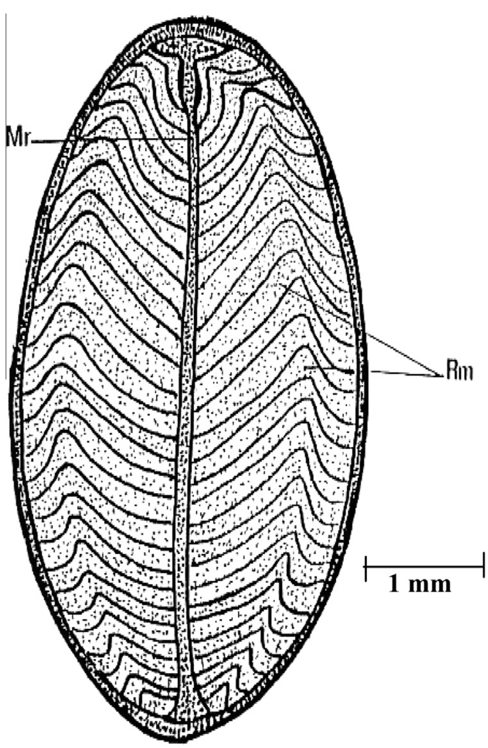

Figure 3 Camera lucida drawing of the olfactory rosette of *A. anguilla*, showing Mr, median raphe; Rm, radial lamellae.

(1909). A long and narrow median raphe is situated in the middle of the rosette extended from its anterior to posterior ends. The olfactory rosette is formed of a great number of thin lamellae, arranged in a feather-like pattern on both sides of the median raphe and attached peripherally to the walls of olfactory chamber (Figs. 3 and 5). Each lamella is composed

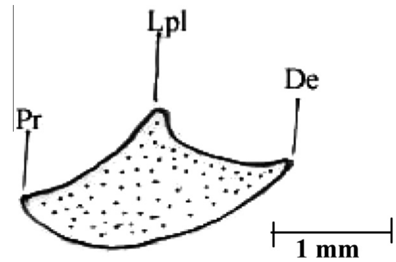

Figure 4 Camera lucida drawing showing the structure of a single lamella of *A.anguilla*, showing Pr, proximal end of lamella; De, distal end of lamella; Lpl, linguiform process of lamella.

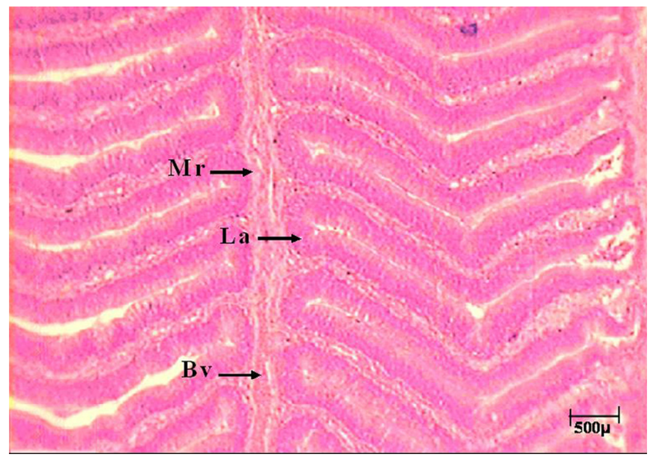

Figure 5 Photomicrograph of L.S of the olfactory rosette of *A.anguilla*, showing Mr, median; La, lamella; Bv, blood vessel (H&E).

of a central or proximal segment attached to the raphe, the peripheral or distal segment is attached to the wall of olfactory chamber and a free well developed linguiform process extends from the distal free margin (Fig. 4). The number of lamellae of the olfactory rosettes increases with the increase of fish length. The olfactory lamellae are large in the middle of the olfactory rosette and decrease in size towards its anterior and posterior ends indicating that new lamellae are added to the rosette at both ends (Fig. 3).

Histological study

(I) Olfactory organs

In the examined fish, each olfactory lamella consisted of an anterior and posterior olfactory epithelia around a central space (the central core). The lamellae are completely covered by the olfactory epithelium except for the region of the median raphe. The olfactory epithelium is thick and demarcated from the central lamellar space by a well developed and usually distinguishable basement membrane (Figs. 5 and 6). The olfactory epithelium consists of the following four types of cells.

(1) Supporting or sustentacular cells (SSP)

These cells form the most superficial layer of the epithelium. They are pseudostratified columnar cells, bearing faintly visible cilia which are connected with basal bodies. The nuclei of these cells are small and lightly stained (Figs. 6 and 7).

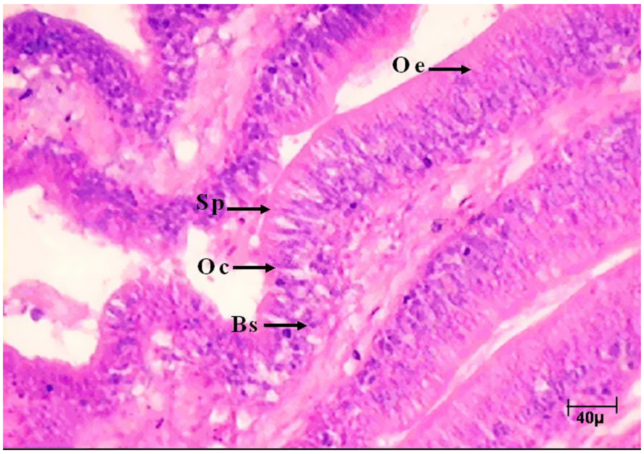

Figure 6 Photomicrograph of L.S of the olfactory rosette of *A.anguilla*, showing the structure of the olfactory epithelium. Oe, olfactory epithelium; Sp, supporting cell; Oc, olfactory receptor cell; Bs, basal cell (H&A).

(2) Olfactory receptor cells (ORC)

The olfactory receptor cells are bipolar neurons, occupying the whole thickness of the olfactory epithelium and constituting the sensory elements of the olfactory epithelium. They are present mainly in between the supporting cells and are differentiated by their elongated or oval and deeply stained nuclei (Figs. 6 and 7).

(3) Basal cells (BS)

These cells are scattered in the deeper part of the olfactory epithelium. They are comparatively smaller than the other cells. They are either cuboidal, oval or rounded and contain distinct nuclei. These cells form a reservoir for the formation of supporting and olfactory receptor cells as they migrate towards the upper part of the olfactory epithelium (Figs. 6 and 7).

(4) Goblet cells (Gb)

These cells are numerous and are scattered in the superficial layer of the olfactory epithelium. They are ovoid or rounded in shape and their nuclei are shifted towards the basal ends (Fig. 7).

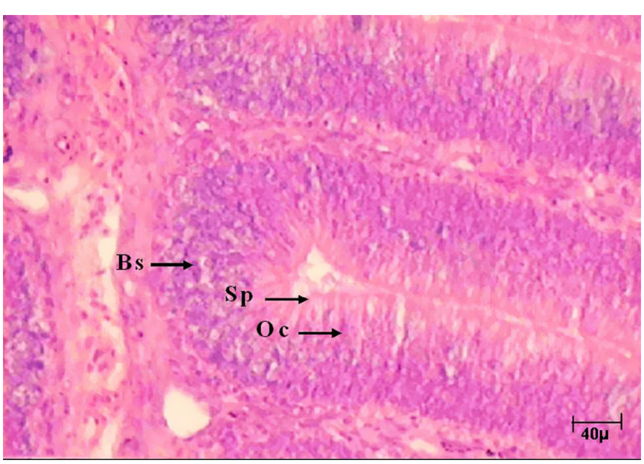

Figure 7 Photomicrograph of L.S of the olfactory rosette of *A.anguilla*, showing the structure of the olfactory epithelium in the neighbourhood of the median raphe. Oc, olfactory receptor cell; Sp, supporting cell; Bs, basal cell (H&E).

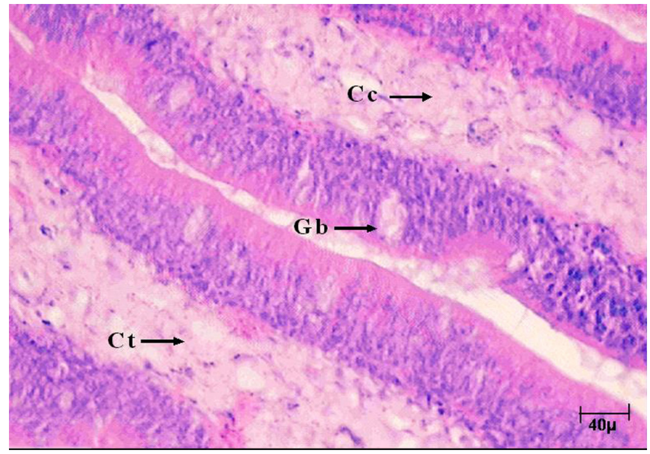

Figure 8 Photomicrograph of L.S of the olfactory rosette of *A.anguilla*, showing the structure of the olfactory epithelium. Cc, central core; Ct, connective tissue; Gb, goblet cell (H&E).

Central core

Central core which is lined by the olfactory epithelium on either side, consists of loosely arranged connective tissue containing nerve fibre and blood vessels (Figs. 8 and 9).

It has been found that in *A. anguilla*, which is a macrosmatic fish, the olfactory epithelium is much folded to increase the dimension of the olfactory area.

(II) The eyes

The retina is the inner sensory (photosensitive) tissue layer of the eye which communicates with the brain through the optic nerve. The structure of retina of the examined fish is identical to that of the other vertebrates. It is composed of ten layers arranged in the following sequence (from outer to inner): pigmented epithelial layer, photoreceptor cell layer, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglionic layer, nerve fibre layer and inner limiting membrane.

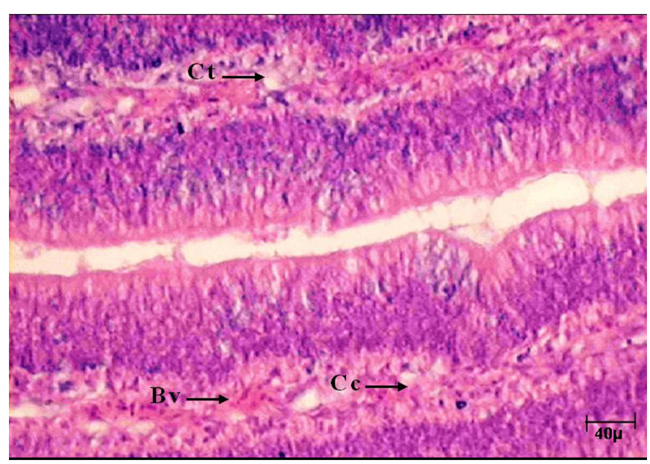

Figure 9 Photomicrograph of L.S of the olfactory rosette of *A.anguilla*, showing the structure of the central core of the olfactory epithelium. Cc, central core; Ct, connective tissue; Bv, blood vessel (H&E).

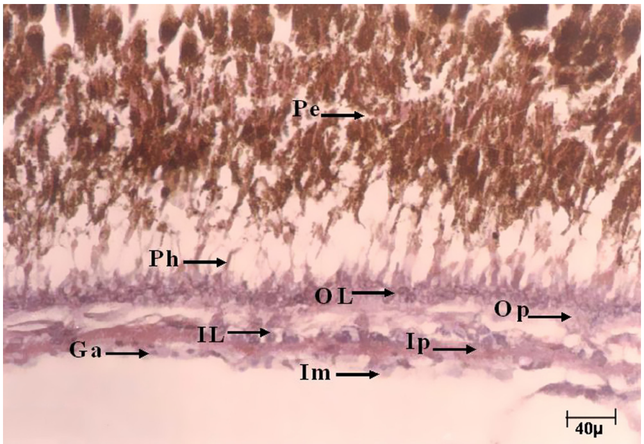

Figure 10 Photomicrograph of T.S of the eye of *A.anguilla*, showing the different layers of retina. Pe, pigmented epithelium; Ph, photoreceptor cell; Ol, outer nuclear layer; IL, inner nuclear layer; Op, outer plexiform layer; Ip, inner plexiform layer; Ga, ganglionic layer; Im, inner limiting membrane (H&E).

The outer wall of the optic cup gives rise to a non-nervous area represented by the pigment epithelium of retina, while its inner wall forms the inner nervous area of the retina.

(A) Pigment epithelium of the retina (Pe)

It consists of columnar epithelial cells containing melanin, which absorbs the light passing through the layers of rods and cones. Although the cells of this layer send out digitiform processes between the photoreceptor cells, there is no actual anatomical connection between the photosensitive and the pigmented layers, except at the head of the optic nerve.

The pigmented epithelium was found to be very thick in *A. anguilla* which is a bottom feeder fish (Fig. 10).

(B) Nervous area of the retina

This area is representing the photosensitive or optical region and made up of the following layers.

(1) Photoreceptor cell layer (Ph)

Generally, there are two types of photoreceptor cells, rods and cones situated just below the pigmented epithelium. The rod cells which are long and slender with a basal nucleus, have low threshold to light stimulation and are effective in dim light. However, the cone cells which are of the same structure as the rod cells, but with an outer cone segment have high threshold to light stimulation and require good illumination to be properly stimulated (Arnold, 1977). Thus, in *A. anguilla* which is a bottom feeder the photoreceptor cell layer is made only of rod cells (Fig. 10).

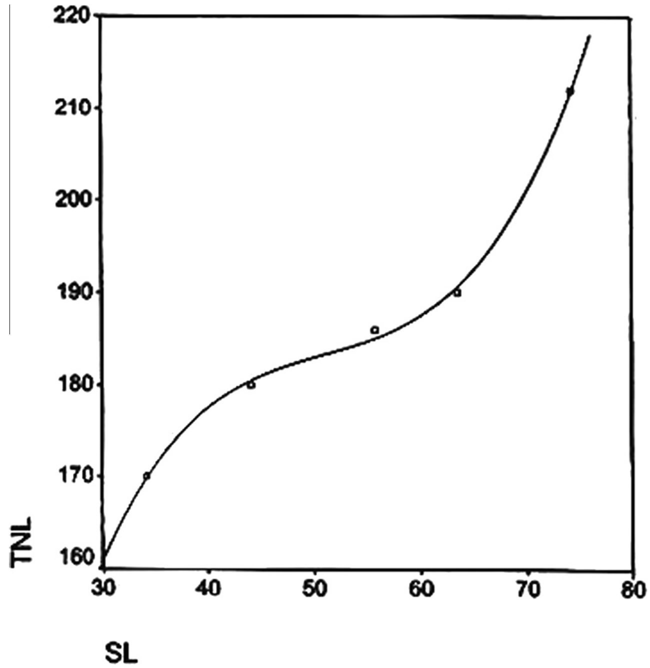

Figure 11 Relationship between number of lamellae and standard body length (SL) of *A. anguilla*.

(2) Outer nuclear layer (Ol)

This layer represents the nuclei of the photoreceptor cells. It seems that the nuclei of the rods are closer to the internal retinal layers.

(3) Inner nuclear layer (Il)

This layer contains the nuclei of several types of neurons, mainly bipolar, amacrine and horizontal cells, as well as Muller's cells. They form a thick middle layer of the retina which transmits impulses from the outer photoreceptors to the inner ganglionic neurons. Outer to the bipolar cells, a thin unicellular continuous layer belonging to the inner nuclear layer forms the outer horizontal cells (Fig. 10).

(4) Outer and inner flexiform layers (Op and Ip)

These two layers form the synaptic relationship between photoreceptor, bipolar and horizontal cells as well as between the bipolar and ganglionic cells, respectively. In the outer plexiform layer, a layer of external horizontal cells is found (Fig. 10).

(5) Ganglionic layer (Ga)

This layer is in the form of a single row of neurons. Their dendrites synapse with the axons of the inner bipolar cells,

Table 1 The average number of olfactory lamellae of *A. anguilla*.

Fish length group	Number of examined fish	Average SL (cm)	Number of olfactory lamellae		Total number of lamellae in both rosettes
			Right	Left	
30–39.9	6	34.2	85	85	170
40–49.9	5	44.1	90	90	180
50–59.9	8	55.8	92	94	186
60–69.9	4	63.5	94	96	190
70–79.9	3	74.3	100	112	212
Average		54.4	92	95	187

Table 2 The average ratio of SO = surface area of the olfactory lamellae and that of SL = standard length of *A. anguilla*.

Fish length group	Number of examined fish	Average SL (cm)	Average SO (cm^2)	Average SO/SL (%)
30–39.9	6	34.2	1.518	4.439
40–49.9	5	44.1	1.818	4.122
50–59.9	8	55.8	2.171	3.891
60–69.9	4	63.5	2.405	3.787
70–79.9	3	74.3	2.733	3.678
Average		54.4	2.129	3.983

while their axons form fibres of the optic nerve. These fibres, emerge from the limiting of the eyeball cavity, collect and extend towards the brain.

(6) Nerve fibre layer

This layer represents the axons of the cells of the overlying ganglionic layer which forms a single row.

(C) Inner limiting membrane (Im)

It is very thin and acts as a basement membrane. It represents a vascular layer interposed between the axons of the ganglionic cells and vitreous humour filling the eyeball.

Morphometric studies

(1) Relationship between the number of olfactory lamellae and standard body length

The total number of the olfactory lamellae in the two rosettes regularly increases with the increase of body length (Table 1). In *A. anguilla*, the total number of the olfactory lamellae in the two rosettes ranged between 170 and 212 with an average number of 187 lamellae, within body length ranging from 30 to 80 cm (Table 1). A curvilinear relationship was obtained (Fig. 11) and can be represented by:

$$TNL = 170 - 0.0027(SL)^2 + 0.001(SL)^3$$

where TNL = Total number of olfactory lamellae, SL = standard fish length.

The two variables are highly correlated (r = 0.964).

(2) Relationship between surface area of the olfactory lamellae and standard body length

In *A. anguilla*, it was found that the average olfactory surface area of lamellae in one rosette (SO) slightly increase as the standard fish length increases (Table 2). A straight line relationship was obtained when the olfactory surface area was plotted against the body length (Fig. 12) and this can be expressed by the following equation:

$$SO = 0.4816 + 0.0303SL$$

where SO = olfactory surface area of lamellae, SL = standard length.

The two variables are highly correlated (r = 1.00).

(3) Relationship between surface area of retina and standard body length

In *A. anguilla*, the surface area of retina was increased with the increase of body length even the percentage ratio of the retinal area to the body length also increases as the fish grows in length (Table 3). A curvilinear relationship was obtained (Fig. 13) and the two variables SR (retinal area) and SL (standard length) are highly correlated (r = 0.983).

(4) Relationship between ecological coefficient (SO/SR%) and standard body length

In *A. anguilla* it was found that the ecological coefficient (SO/SR%) which is the percentage ratio of the olfactory surface area to the retinal area increased with the increase of the fish length (Table 4). A curvilinear relationship was obtained (Fig. 14) and can be expressed by:

$$SO/SR = 1391.51 - 21.644SL + 0.1196(SL)^2$$

where SO/SR = ecological coefficient, SL = standard length.

The two variables are highly correlated (0.896).

The average olfactory surface area of lamellae was found to be 590.967% of retinal area (Table 4). This indicates that the rate of growth in the olfactory area is much more than the rate of growth in retinal area.

Discussion

The olfactory and visual systems of fishes play a major role in fish behaviour, however in many animal groups, olfaction may be one of the senses by which they compensate their reduced visual system. Accordingly, Popova (1967) divided fishes into two groups on the basis of their method of finding and procuring food; being diurnal and nocturnal. In the former group, the

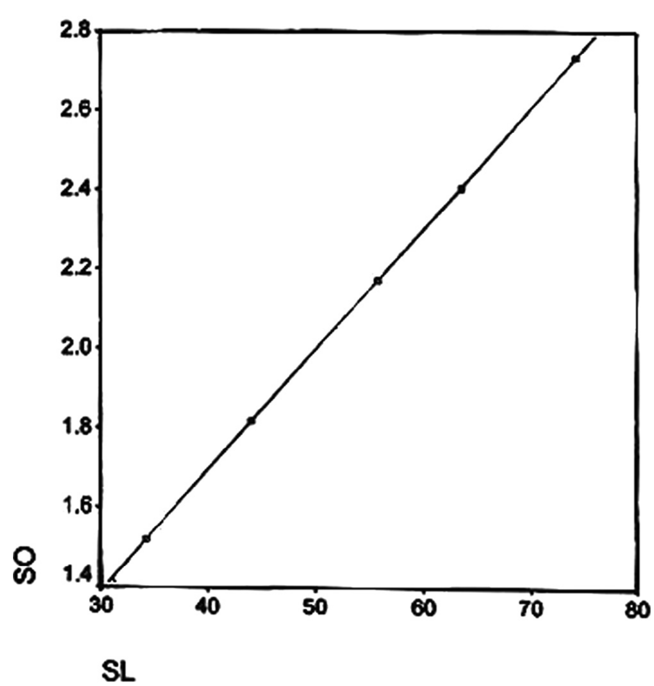

Figure 12 Relationship between surface area of the olfactory lamellae (SO) and standard body length (SL) of *A. anguilla*.

Table 3 The average percentage ratios of SR = area of retina and that of SL = standard length of *A. anguilla*.

Fish length group	Number of examined fish	Average SL (cm)	Average SR (cm^2)	Average SR/SL (%)
30–39.9	6	34.2	0.185	0.541
40–49.9	5	44.1	0.305	0.692
50–59.9	8	55.8	0.358	0.642
60–69.9	4	63.5	0.479	0.754
70–79.9	3	74.3	0.636	0.856
Average		54.4	0.393	0.697

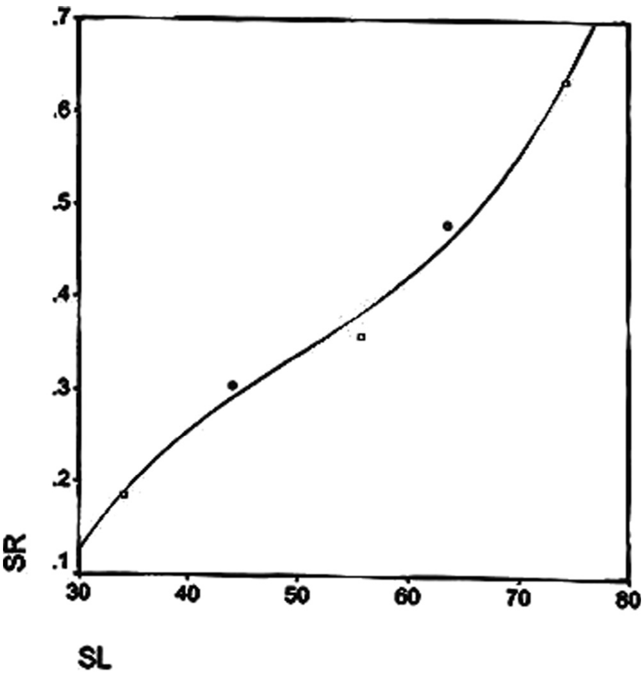

Figure 13 Relationship between area of retina (SR) and standard body length (SL) of *A. anguilla*.

vision plays the main role in capturing prey, whereas fishes of the latter group apply the senses of smell, touch and lateral line organs as the main ones.

In *A. anguilla*, the olfactory organs are represented by a pair of olfactory chambers, each opens to the exterior by two nostrils, an anterior inlet and posterior outlet. The water flows into the olfactory chamber via the inlet nostril and leaves through outlet one as reported also by El-Attar et al. (2006) in *Bagrus bayad* and El-Attar and Al-Zahaby (2010) in silver carp. Early Pipping (1926) mentioned that the number of nares plays a role for the effectiveness of olfactory organ. She found that *Casterostens aculeatus* has a monotrematous olfactory organ, i.e. with a single naris. It does not react to food odors and perhaps the organ is used for detection of other chemical stimuli than those provoked by food.

The olfactory rosette of *A. anguilla* is oval and thus it belongs to type II of Bateson (1889) or column II of Burne (1909) and in accordance with that of *Bagrus bayad* (El-Attar et al., 1999, 2006). In the species studied, the number of olfactory lamellae and average surface area of lamella increased with the fish body length. Similar findings were also observed in other fishes by El-Attar, 1990; Ismail, 1991; and El-Attar et al., 1999.

The structure of the olfactory epithelium in *A. anguilla* reveals the presence of olfactory receptors, as well as supporting, basal and goblet cells. This fact was noticed in different fishes by Ismail, 1991; and El-Attar et al., 1999, 2006. The same authors also illustrated that the basal cells migrate outwards to form receptor and supporting cells. This finding was noticed in *Notopterus notopterus* by Goel (1978).

In *A. anguilla*, the surface area of the olfactory lamellae was found to be about 590.9% of the retinal area. Thus, the olfactory organs are well developed in the bottom feeder *A. anguilla* than the eyes and so, the olfaction plays an important role in their feeding habits. Therefore, the fish is macrosmatic and belongs to Teichmann (1954) group III "nose-fishes". This finding is in accordance with that of *Wallago atto* (Ojha and Kapoor, 1972), *Cynoglossus oligolepsi* (Kapoor and Ojha, 1973), *Clarias lazera* (El-Attar and El-Agamy, 1989) and *Alestes nurse*, *Oreochromis niloticus* and *Bagrus bayad* (El-Attar et al., 1999, 2006).

The pigmented epithelium of the retina represents the non-nervous area. It is very thick in *A. anguilla* which is a bottom feeder. Arnold (1977) attributed the thickness of this layer to the scarcity of light in the environment of such fishes. Light that passes beyond the rods and cones is reflected back to them where it can stimulate these receptors again. Donatti and Fanta (2002) also stated that, the retinal pigment epithelium photophors are optimally exposed to incoming light in the dark in the case of rods and under light conditions in the case of cones. This capacity might be important to enable the fish to

Table 4 The average percentage ratios of SO = surface area of olfactory lamellae and that of SR = area of retina (ecological coefficient) of *A. anguilla*.

Fish length group	Number of examined fish	Average SL (cm)	Average SR (cm^2)	Average SO (cm^2)	Average SO/SR (%)
30–39.9	6	34.2	0.185	1.518	820.541
40–49.9	5	44.1	0.305	1.818	596.066
50–59.9	8	55.8	0.358	2.171	606.425
60–69.9	4	63.5	0.479	2.405	502.088
70–79.9	3	74.3	0.636	2.733	429.717
Average		54.4	0.393	2.129	590.967

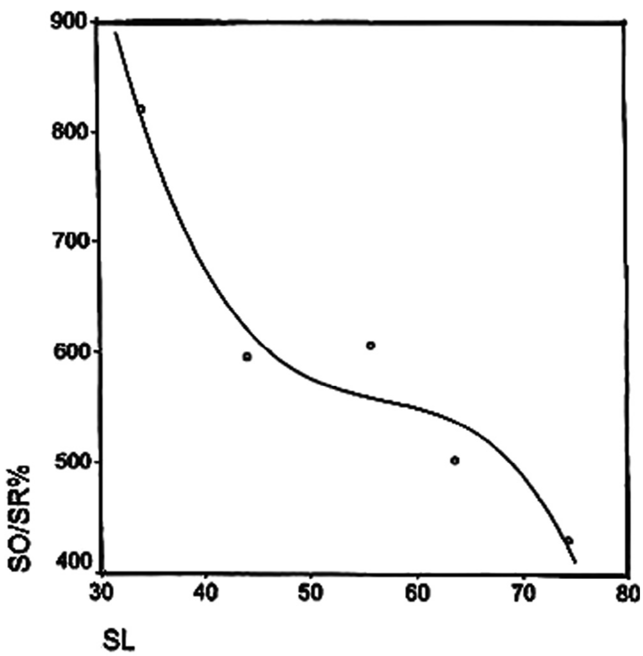

Figure 14 Relationship between ecological coefficient (SO/SR%) and standard body length (SL) of *A. anguilla*.

find their prey under light and dark conditions or even when weather changes, resulting in sudden changes in light conditions.

The photoreceptor cell layer is usually composed of rods and cones of retina. In *A. angullia*, as a bottom feeder, this layer is made up only of rod cells. This finding is in accordance with that of *Bagrus bayad* (El-Attar et al., 1999). Also, they found that in *Alestes nurse*, as a surface feeder, the layer is made up only of cone cells. But in *Oreochromis niloticus*, which is mid-water and surface dweller, this layer is essentially composed of cone cells and rarely rod cells are observed. This difference in the structure of photoreceptor layer in *A. anguilla* can be attributed to the fact that cone cells have a high threshold to light stimulation and require good illumination. But, rod cells have a low threshold to light stimulation and are effective in dim light, as in case of bottom feeder *A. anguilla*.

In conclusion, the present investigation revealed a correlation between the feeding habits of freshwater teleost fish *A. anguilla* and the structure of their sense organs, as a bottom feeder depends entirely on the olfaction.

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